LESSON 13:
MICROPROPAGATION METHODS

Introduction

Among the various applications of plant tissue culture, micropropagation of plant species has attained the status of large plant based industry. The developments in the study of various aspects of plant growth and differentiation were rapid during 1960s and 70s. The technique of culturing plants became a wide subject embracing morphology, physiology, biochemistry, molecular biology and genetic engineering. The rapid multiplication of orchids through protocorm culture by Morel (1960) lead to the establishment of the first tissue culture based industry for rapid multiplication of orchids.

Multiplication of plants through plant tissue culture can be achieved by any of the following methods depending upon the objectives. The basic concept is to achieve rapid multiplication without creating unwanted somaclonal variation. Therefore, axillary and adventitious budding and somatic embryogenesis are most frequently used methods of micropropagation.

What is Micropropagation?

Micropropagation is defined as production of miniature planting material [seeds (somatic embryos) or plantlets] in large number by vegetative multiplication through regeneration.

Various Approaches used for Micropropagation

A. Axillary Budding

Development of shoots from pre-existing meristems on nodal region ensures genetic stability of the regenerants and is termed as axillary budding. This is the most reliable method of in vitro propagation producing about 90% of the current production of the micropropagated plants. Propagation of long (life) cycle plants such as forest and fruit trees is multiplied mainly through this method. This is also the principal method of propagation of plants at industrial level where high standards of homogeneity are to be maintained e.g., strawberry, rose, potato, artichoke, asparagus, pineapple, banana etc. In this method, the variation developed during multiplication does not exceed the percentage of variation found in the natural population prepared by conventional methods.

B. Adventitious Budding

De novo formation of adventitious buds (not from pre-existing meristems) may occur directly from the tissues of the explant e.g. from leaf petiole or root segments, from internodal region of stem explant (Fig.1). This method offers the required level of genetic stability in the regenerated plants except for chimeral plants such as Saintpaulia and Begonia. In some cases adventitious shoots form indirectly from callus, e.g., in Ficus lyrata and Anthurium andreanum. Since calli are unorganized structures, they are prone to nuclear unstability during rapid cell divisions. Therefore, chances of genetic unstability increase in such cases where intermediate callus is produced.

C. Somatic Embryo Development

Somatic embryos occur either directly on explants or more frequently in callus cultures (indirect somatic embryogenesis). Indirect embryogenesis involves callus or cell cultures and hence will lead to the production of somaclonal variation in the regenerated plant population. This method does not ensure genetic stability. However, somatic embryogenesis in cell cultures is very useful for developing scale-up technology using bioreactor and in the production of synthetic seeds through encapsulation. This is not possible with any other method. Therefore, this method can be used when very high standards of genetic stability are not required.

Thus, in practice, most micropropagation is achieved by maintaining organized tissues by the multiplication of meristems and axillary buds.

Advantages of Biotechnology

Plant tissue culture offers several advantages over conventional propagation methods, vegetative and sexual, for large-scale micropropagation. These are:

1. Shoot multiplication can be achieved in small space because miniature plantlets are produced. Each flask can accommodate hundreds of plantlets. In case of somatic embryogenesis, this number is enormous producing 20,000 to 50,000 embryos per litre of medium (small space).
2. Propagation is carried out under sterile conditions. No damage is caused due to insects and diseases, and plantlets produced are free from microbes (pathogens).
3. In case, virus-free material is used (even through virus elimination by meristem culture), a large number of virus-free plants can be obtained (virus-free plants).
4. Plant tissue culture is carried out under defined conditions of environmental, nutritional and tissue system, therefore, it is a highly reproducible system under the defined set of conditions (controlled conditions, reproducibility).
5. This production is unaffected by seasonal variations as uniform conditions are maintained (no seasonal effect).
6. No care is required between two subcultures as compared to conventional vegetative propagation systems like watering, weeding (less care).
7. Small glass house space is required because of miniature size of plant lets (small glass house space).
8. Mother plant or genotype of stock plant can be stored and maintained in vitro without dam age to environmental factors and stock plants.
9. The plants which are difficult to propagate vegetatively by conventional methods can be propagated by this method (difficult to root plants).
10. It is possible to mechanize whole process of vegetative propagation for large-scale plantations (mechanization).

11. Miniature storage organs (tubers, corm, tuberous roots) can be produced for genotype storage and subsequent plantations (Germplasm storage).
12. Being sterile, transport across countries is permissible without difficulties (transport across countries does not require phytosanitory regulations).

Disadvantages of Biotechnology

Though, there are several advantages, there are also certain disadvantages with the system.

These are:

I. Micropropagation methods through use of tissue culture involve capital intensive expensive materials like autoclave, laminar air flow bench, controlled culture rooms etc. (capital intensive).
2. This is a technically skilled work; knowledge about material, techniques and decision mak ing (during subculture and multiplication of propagule) are required in the personnel (skilled type work).
3. Contamination is a serious threat and cause severe damage to material and add substantively lot to the cost of production, affects time schedule delivery of the material (contamination).
4. Specific conditions of micropropagation, rooting, and hardening may be required. Therefore, each material requires separate research methods (No uniform standard method).
5. Small delicate plantlets are produced, which take longer initial time to grow.
6. Genetic stability is doubtful in certain methods.
7. It is a capital intensive industry, if plants are produced in small number, they cost too much, otherwise also cost is a major factor for the production and sale of tissue culture raised plants (high cost of plantlets).

Micropropagation Methods

The information and knowledge about botany, reproductive biology, geographic distribution and growth seasons are required to obtain plant material in healthy state for optimal growth. The stages involved in the micropropagation of a given plant genotype are given in the following paragraphs. These stages are used irrespective of the approach (axillary budding or somatic embryogenesis) used for multiplication of the species. Little modifications are possible with individual case to adjust the methodology.

The schematic layout of the method is given in Fig.1.

Stage I. Selection and Establishment of Aseptic Cultures

This step is concerned with selection of typical, healthy, disease free mother plants. If necessary, test for virus presence is carried out and if found positive it is eliminated. Selection of plant is followed by preparation of explants, surface sterilization and transfer to appropriate media; sterilization is carried out through soaking in a calcium hypochlorite solution; details of sterilization are described in the previous Lesson.

The objective of this stage is to attain an aseptic culture of the plant in question, the culture may be initiated from any plant part and callus or organ formation can be achieved.

Nutrient medium is selected either from the existing media or may be evolved empirically. In any case, medium should support the rapid proliferation of the explants. Influence of the medium components on regeneration is discussed in the previous Lessons. The regeneration media for shoot formation should not contain 2,4-0 but 2,4-D may be used for somatic embryogenesis, particularly for monocots. Usually shoot forming cultures are illuminated for 12-16 h (l000-2000 lux).

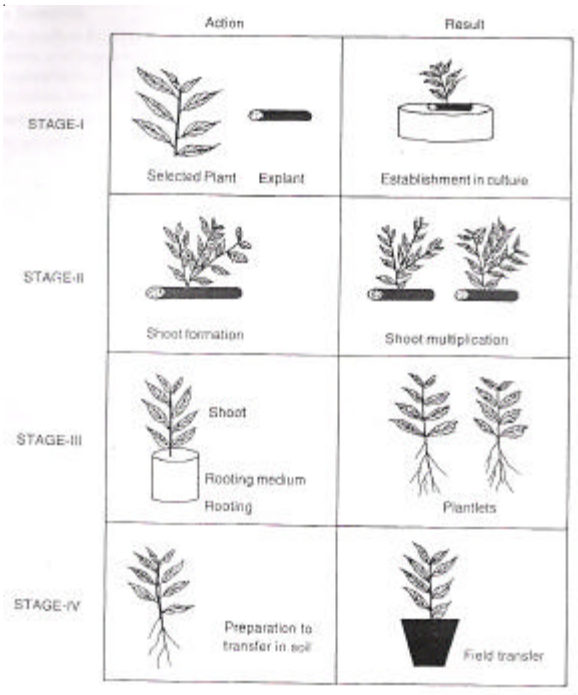

Fig.1 . Micropropagation method-stages to obtain in vitro multiplication.

Stage II. Multiplication of Propagule

This stage is concerned with rapid multiplication of the regenerative system for obtaining large number of shoots. To achieve this, medium and tissue factors are optimized empirically. In vitro produced shoots are used as explants to produce more shoots. The shoots may be obtained by any of the approaches mentioned above (axillary, adventitious or embryogenesis). Orchids are multiplied by proto corm formation.

Normally, medium for stage I and II is same, but minor changes may be made in the concentration of cytokinins or nitrogen to increase the shoot proliferation. Repetitive use of in vitro produced explants and subcultures of the propagule on the same medium enhance the shoot proliferation and produce adventitious shoots. All the culture conditions are the same as used for stage-I.

Stage III. Plantlets Regeneration

Plantlets are produced through rooting of isolated shoots or germination of somatic embryos. For this purpose, shoots of a proper length or age are required, which may be produced by the medium combinations. Shoots are separated manually from clusters and transferred on a rooting medium containing an auxin. A pulse-treatment with high concentration of an auxin and transfer on hormone free medium is preferred over continuous growth of shoots on an auxin containing medium. The difficult to root species, particularly tree species, may require modifications in nutritional or environmental conditions such as activated charcoal, high temperature or liquid medium with filter paper bridge for high rooting. Cultures for rooting are placed in low light intensity (about 200-1000 lux, compared to stage II) or the lower portion of the culture tubes is covered with black paper to facilitate root induction. Low salt strength of the rooting medium facilitates the rooting. In some herbaceous plants, in vitro produced shoots are pulse-treated with auxin and transferred directly to pot mixture, there they root, e.g., in Chrysanthemum.

Stage IV. Preparation and Transfer to Field

This stage is concerned with transfer of plantlets in pots, their hardening and establishment in soil. A successful tissue culture method of propagation must result in re- establishment in soil of a high frequency of the tissue culture derived plants. Stage IV is intended to prepare the propagule for their successful transfer to soil. Hardening of plants imparts some tolerance to moisture stress and plants become autotrophic from heterotrophic condition. When storage organs are formed on plantlets, their establishment in soil becomes easier e.g., tubers formation in Ceropegia, tuberous roots formation in Curculigo and Chlorophytum, bulblets formation in Allium spp. Bulbs, corms and tubers may undergo dormancy when transferred from test tube to soil. Such tuberous organs may require the chilling treatment to germinate. When plantlets are taken out from the vessels, adhering agar is removed by careful washing with running tap water and plantlets are transferred in a soil: vermiculate (1:2) mixture and placed in mist-house under high humidity conditions. Plantlets are irrigated with a dilute nutrient solution or pure water. In laboratory, plantlets are covered with glass beakers. Plantlets are exposed to decreasing humidity by slowly exposing the plant (by removing glass covers) or reducing the mist period in the glass house. Plantlets develop cuticle and their stomata start functioning. During this period high light intensity may also be provided (3000-10000 lux). .

Hardened plants are then transferred to glass or poly-houses (tent like structures are erected by mounting polythene or polycabonate sheets on metal frame support) with normal environmental conditions. Plants are irrigated frequently and their growth and variation are monitored regularly. Plants may be kept upto flowering (flowering plants) or transferred to fields (plantation crops) after 4 to 6 weeks of acclimatization.

Clonal Multiplication of Woody Species

Woody perennials comprise valuable crops such as fruit and nut trees, plantation species (palms of various types, rubber, coffee, tea etc.), timbers (hardwood and softwood) and important trees of social forestry. Due to the long-term life cycle of these tree crops, progress in the improvement of woody perennials through the application of conventional methods has been extremely slow. The difficulty in rooting the cuttings from elite tree species (Quercus, Fagus, Eucalyptus and most conifers) has further complicated the process of their clonal propagation. According to Jones (1983), vegetative propagation of many monocotyledonous palms and forest species is virtually impossible. Conventionally, forests are multiplied by the growth of natural seedlings and by collection of seeds from randomly pollinated elite trees. Cryptomeria japonica is an exception which has been clonally propagated for centuries in Japan. Recently, attempts were made to establish clones of Norway spruce (Picea abies cv.) on a large scale in the forests of Sweden and the former Federal Republic of Germany (Paranjothy et at 1990).

Hardwoods and Fruit Trees

During the past many years considerable success has been achieved with respect to cloning of temperate fruit species and other trees through tissue culture. The response of more forest trees, as Populus, Eucalyptus and Tectona, to micropropagation has been highly positive. Tissue culture of nut trees also holds some promise (Zimmerman 1986). Recent success in tree tissue culture is the controlled flowering of in vitro propagated bamboo (Nadgauda et al. 1990). Explants that have shown positive response in cultures for regeneration, however, are largely restricted to juvenile material. Clonal multiplication of woody perennials in vitro on a commercial scale requires enormous efforts directed towards establishing cultures from adult explants. Further, effective treatments to induce efficient rooting of in vitro multiplied shoots and quality improvement of somatic embryos to achieve high frequency conversion to plantlets must be found. Shoot tips from seedlings of Citrus, jackfruit and black plums have readily responded in culture. Immature embryos of avocado have proved equally effective clonal materials in cultures. Since nucellar seedlings are naturally rejuvenated clones, they have been utilised as stock plants for micropropagation of several polyembryonic Citrus cultivars and mangosteens (Barlass and Skene 1986, Goh et al. 1988). Monoembryonic species of Citrus, varieties of mango, loquat and Syzygium spp. regenerate by somatic embryogenesis using nucellar explants (Litz et al. 1986). Also, many tropical and subtropical fruit crops are reported to regenerate from somatic embryos induced on explants from mature trees.

The nature of the explant, its orientation on the culture medium and genotype influence the response of adult materials in vitro. Shoot tips or nodal segments from tropical adult trees are reliable sources of explants. These explants can be excised from newly developed vegetative buds of jackfruit (Amin 1987) at the base of the main stem during the onset of flowering or during vigorous vegetative growth of guava (Jaiswal and Amin 1987). In avocado, hard pruning of the main stem stimulates the growth of lateral buds, thus providing enough meristematic material for clonal propagation (Pliego-Alfaro et at 1987). The conditioning of stock plants improves the culture response of shoot tips and nodal segments and so do warm and humid months (guava; Jaiswal and Amin 1987). Sometimes horizontal

placement of shoot-tip explants on the medium may prove superior to vertical orientation.

Tropical fruit species which could be commercially propagated are banana, papaya and pineapple. The planting of extensive orchards of vegetatively propagated clones of some tropical and subtropical fruits has remained limited due to loss through pathogen attacks. Most Citrus-producing countries have established programmes for release of clonal material that has been indexed for freedom from Citrus tristeza virus after micrografting of meristems onto seedling rootstocks in vitro (Blitters et al. 1970). Other diseases of fruit trees, such as root rot of avocado caused by Phytophthora cinnamoni, could be controlled if clonally propagated disease-resistant rootstocks were readily available.

Softwoods

Gymnosperms account for most of the world's produce of timber (softwood). Clonal propagation of gymnospermous species is desirable because of their long lifespan and open pollination. Vegetative multiplication by cuttings has drawbacks as cuttings lack the vigour of seedlings and their growth declines with increasing age of the ortet. The in vitro techniques are expected to help overcome these problems and regenerate plants at faster rates.

Tissue culture studies with gymnosperms commenced with Gautheret (1934) who cultured cambial tissues of Pinus pinaster and Abies alba. La Rue (1936) cultured embryos of several gymnosperms into normal-looking seedlings. Buds were also obtained from callus cultures of Sequoia sempervirens (Ball 1950). Despite these early successes little headway was made on tissue culture of gymnosperms until 1975 when reports appeared on complete regeneration of Pinus palustris plantlets (Sommer et al. 1975). Since then plantlets have been obtained via somatic embryogenesis and organogenesis from about 35 gymnosperm species (Hakman et al. 1985, Dunstan 1988).

A large number of adventitious buds can be induced on embryonal explants of gymnosperms. However, the number of plants finally established in soil is extremely low because of losses during successive stages of development, rooting, or transplantation. Yet, large-scale plantation out of embryo or cotyledonary plants is possible in Pinus radiata, P. taeda, P. pinaster, Pseudotsuga menziesii and Sequoia sempervirens. Plagiotropic growth is seen in many species (e.g., Pinus taeda, P. radiata, Pseudotsuga menziesii and Cunninghamia lanceolate; see Paranjothy et al. 1990). Plantlets of these species have relatively less-field-survival than orthotropic plantlets. In Douglas-fir, plagiotropic plantlets recover faster in soil and become orthotropic sooner than cuttings (Abo-EI-Nil 1987, Amerson et al. 1988). So far, only limited success has been achieved with micropropagation of plants from adult tissues. However, the possibility of clonally multiplying older trees and establishing field trials of in vitro derived plants has been demonstrated in species such as Sequoia sempervirens, Pinus pinaster and P. radiata.

Induction of somatic embryogenesis using immature embryo cultures of Picea abies (Hakman et al. 1985) and female gametophyte of Larix decidua (Nagmani and Bonga 1985) has been a major breakthrough in the tissue culture of gymnospermous species yielding softwoods. Other reports of similar nature are of Pseudotsuga menziesii (Durzan and Gupta 1987), Larix sp. (Klimaszewska 1989), Pinus taeda (Gupta and Durzan 1987) and P. strobus (Finer et al. 1989). In all these cases precotyledonary embryos (2-3 weeks after fertilisation) were used since older embryos yielded non-embryogenic calli. The possibility of obtaining somatic embryos from a mature zygotic embryo culture has been demonstrated in Picea abies (Von Arnold and Hakman 1986), P. glauca (Kartha et al. 1988) and Pinus lambertiana (Gupta and Durzan 1986). Well-developed somatic embryos have also been obtained from protoplasts derived from embryogenic calli of Pinus taeda (Gupta and Durzan 1987) and Picea glauca (Attree et al. 1987).

Somatic embryogenesis involves a callus phase which can be induced in the presence of a high auxin to cytokinin ratio, but the maturation of these embroys requires the removal of phytohormones or application of ABA (Dunstan et al. 1988). This results in poor recovery of plantlets from somatic embryos of these species and, therefore, is an impediment to micropropagation of gymnosperms. Another limiting step in clonal propagation of taxa of this group is the non-repeatability of results particularly regarding the tissue culture of conifers, which may be due to an inherent variable genetic constitution within the cells of the explant.

Alternatively, plantlets may be regenerated directly from zygotic embryos of various species of Pinus and clonally propagated.

Some Technical Problems Encountered in Micropropagation

Contamination of Cultures

During large-scale micropropagation of some plants certain types of slowgrowing microbial (bacterial) contaminants persist even after initial surface sterilisation of explants. Such contaminants (Pseudomonas sp., Erwinia sp. and Bacillus sp.) may persist for many generations without being noticed and cause reduction in vigour or chlorosis in propagated plantlets (Knauss and Miller 1978). Addition of antibiotics or fungicides to the culture medium may control the infection by microbial contaminants but for propagation of plants normally infected with latent or symptomless types of viruses or mycoplasmas, it is necessary to maintain stock of plants of them which are disease-free.

Browning of the Medium

A problem more frequently associated with micropropagation of woody perennials is the accumulation of inhibiting substances in the growth medium during initiation of cultures. Explants from adult tissues of these species often produce excessive amounts of phenolic substances which turn the medium dark brown. Such a medium is toxic to the tissues and inhibits their growth. Quick transfer of explants to a fresh medium at short intervals could alleviate this problem in orchid bud cultures. Browning of the medium may also be prevented by dissecting explant tissues under the surface of liquids or by incorporating ascorbic acid or citric acid in culture media. For teak tissue cultures, polyvinyl pyrrolidone has proved an effective amendment to the culture medium. However, it may

be emphasised that phenolic compounds such as phloroglucinol (PG) have been found essential for shoot multiplication and rooting in a number of rosaceous cultivars (Jones 1983). PG (found in the xylem sap of apple) is known to promote shoot multiplication and rooting in Pissardi plum and Cinchona (see Bhojwani and Razdan 1983, Mantell et al. 1985).

Applications of Micropropagation

The technique of micropropagation, an alternative to conventional metnods of vegetative propagation, is applied with the objective of enhancing the rate of multiplication. Through tissue culture over a million plants can be grown from a small, even microscopic, piece of plant tissue within 12 months. Such a prolific rate of multiplication cannot be expected by any of the in vivo methods of clonal propagation. Further the advantage in propagation through tissue culture is that shoot multiplication usually has a short cycle (2-6 weeks) and each cycle results in logarithmic increase in the number of shoots. Additionally, tissue cultures give propagules such as minitubers or minicorms for plant multiplication throughout the year irrespective of the season. The small size of the propagules and their ability to proliferate in a soil-free environment facilitates their storage on a large scale and also allows their large-scale dissemination by suitable means of transport across international boundaries. Using these methods stocks of germplasm can be maintained for many years.

Clonal propagation in vitro appears to have a permanent advantage in cases in which serious problems with disease occur. This is because of the fact that through in vitro methods more pathogen-free plants can be raised and maintained economically. Multiplication by cloning dioecious species is extremely important when the seed progeny yields 50% males and 50% females and plants of one of the sexes are desired commerically. For example, male plants of Asparagus officinalis are more valuable than the female plants of this species. Clonal propagation of male Asparagus by stem cuttings has not been successful but can be achieved through tissue culture. On the contrary, in seed-raised orchards, micropropagating female plants would save the losses suffered due to discarding a large nu.mber of naturally arising male plants.

A major advantage of micropropagation happens to be the minimum growing space required in commercial nurseries. Several thousand million plants can be maintained inside the culture vials on a shelf space built into a room of about 3m x 3m x 5m. This makes possible the propagation of clones on a commercial scale for large number of horticultural species (African violet, banana, eucalyptus, ferns, orchids, gloxinia, gerbera and rhododendrons) in a single nursery.

Although commercial micropropagation has been possible for quite a number of plants there are many tree crops, including gymnosperms, that cannot be multiplied under both in vivo and in vitro techniques. Considerable research is needed to overcome this limitation. In systems in which aseptic cultures have been established, in vitro propagation through many generations may lead to bulking up of off-types rather than clones. This problem could be offset by a conservative approach, namely adopting enhanced axillary branching. Another approach would be to not go beyond the fourth cycle and to raise only a few thousand plants from a selected explant. Generally, during repeated cycles of in vitro shoot multiplication a percentage of cultures show water-soaked or almost translucent leaves. Such shoots exhibit a decline in rate of growth and may ultimately die. This phenomenon, called vitrification, may be prevented in shoot cultures of globe artichoke (Cyana scolymus) by raising the agar concentration from 0.6% to 1 % in the culture medium. However, this type of treatment is likely to drastically reduce the rate of propagation for most of the tissues.

In conclusion, the investment in commercial tissue culture business will depend to a large extent on cost of the laboratory set-up, type of plant to be propagated and the skill involved. The production cost in developing countries, such as India, would be low in view of the fact that 63% components involve manpower and labour charges in these countries are generally low. These countries could provide an incentive in attracting alien investment (particularly from developed countries) in establishing laboratories for commercial plant micropropagation.

It is advisable that a commercial nursery man start with those crop species for which published methods are available. Also it is essential that growers have some training in tissue culture and plant husbandry for which short courses could be organised from time to time.

Commercial Aspects

There are now many commercial companies, in India, third world and developed countries, producing millions of plantlets through micropropagation. A few selected genera micropropagated commercially in India are enlisted in Table.1. In the Netherlands alone, where there is an efficient pot plants and glass house industry, there are ten companies each producing ten million plants per year. Similar micropropagation companies exist everywhere and some are specializing in only one type of plant e.g., for ferns or gerberas.

In micropropagation companies, facilities exist for simultaneous inoculation of large volume of plant material. For this purpose, large media rooms with precisely controlled medium formulations are used. Large number of laminar flow benches and large growth chambers are present there. Inoculation chambers and growth rooms are clean areas and sterility is strictly maintained. The secondary multiplication of easy to root flowering plants may be carried out in vivo by rooting of the stem cuttings. This reduces the cost and saves time in multiplication.

Large glass houses and green houses are essential components of micro propagation industry. Hardening and acclimatization of delicate in vitro raised plantlets is carried out in these glass houses. Nowadays chamber made of polycarbonate and polypropylene sheets are used for creating large working place. These houses are fitted with mist and fog generating units with cyclic auto-regulation. Light is provided through proper light sources.

Table.1. Plant Tissue Culture Technologies for large-scale production and commercialization

I. FOREST TREES

- Anogeissus pendula
- Bamboo
- Boswellia serrata
- Eucalypts
- Neem
- Poplars
- Salvadora
- Teak
- Wrightia arborea

II. HORTICULTURE CROPS

- Banana
- Citrus
- Potato
- Strawberry
- Sugarcane

III. PLANTATION CROPS

- Black Pepper
- Cardamom
- Coffee
- Ginger
- Tea
- Turmeric

IV. MISCELLANEOUS

- Phragmites

Various industries are involved in micropropagation business in India also. Tissue culture is an important tool of biotechnology which can be used to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. The Task Force on Plant Tissue Culture set up by the Department of Biotechnology discussed the need for initiating specific programmes for different plant species. Thus, a number of research and development projects were supported for perfecting complete protocols of important plant species – Forest Trees, Horticulture Crops and Plantation Crops. The main objective of these projects was to develop a package of technology for production of large quantity of planting material of improved varieties. Two Tissue Culture Pilot Plant facilities were also set up for large-scale production of elite planting material with the main objective of demonstrating the field performance of tissue culture raised plants of forest trees. Each Pilot Plant has a production capacity of 1.0 million plantlets/annum. Hardening Units have been established in different agroclimatic regions.

Plant tissue culture technology has been commercialized and many companies in India are producing large quantities of planting materials especially ornamentals. Commercialization of technology requires scaling up and field evaluation before transfer to the industry. The Department of Biotechnology supported projects successfully standardized complete protocols and processes for a number of economically important plant species. These protocols have been scaled up at the Hardening Units and Tissue Culture Pilot Plants. Tissue Culture raised plants have also been transferred to the field and their performance evaluated. The Pilot Plants have produced nearly 5.0 million tissue culture raised plantlets, especially forest trees and field planted covering an area of approx. 4500 ha.

Genetic fidelity testing of tissue culture raised plants were carried out at Botany Department, Delhi University, Delhi, using molecular markers.

Two Tissue Culture Pilot Plants were converted to Micropropagation Technology Parks (MTP) to provide an effective platform for transfer of the proven technologies to the entrepreneurs. The MTP's have optimized a large number of technologies and also transferred to the industry.

Conclusion

Tissue culture methods offer an alternative means of plant vegetative propagation. Clonal propagation through tissue culture (popularly called micropropagation) can be achieved in a short time and space. Thus, it is possible to produce plants in large numbers starting from a single individual. Micropropagation has, therefore, found wide commercial application e.g., in orchid propagation. Since then, several crop species have been micropropagated and recipes are now available which can be adopted by growers trained in aseptic manipulations and plant husbandry.

Questions

1. Describe the micropropagation methods for the multiplication of flowering plants.
2. Write short notes on the following:
   a. Various approaches used for micropropagation
   b. Commercial aspects
   c. Advantages and disadvantages of micropropagation.

Note